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**NASAL CALCITONIN FORMULATIONS****RELATED APPLICATIONS**

This application is based upon and claims priority  
of U.S. Provisional Application No. 60/180,241, filed  
5 February 4, 2000, which is hereby incorporated by  
reference.

**BACKGROUND OF THE INVENTION****FIELD OF THE INVENTION**

The present invention relates to an intranasal  
10 pharmaceutical compositions comprising calcitonin as an  
active ingredient and specific concentrations of citric  
acid or a salt thereof as a stabilizer and absorption  
enhancer.

**DESCRIPTION OF THE RELATED ART**

15 Calcitonins are a class of polypeptide hormones that  
are used in the treatment of a variety of conditions  
including osteoporosis, Paget's disease and malignant  
hypercalcemia. They are composed of amino acids and have  
been extracted from a number of sources including salmon,  
20 porcine, eel and human. Calcitonins with amino acid  
sequences identical to the natural forms have been

produced by chemical synthesis as well as by recombinant technology.

Given their size and chemical composition,  
5 calcitonins were originally administered by subcutaneous  
or intramuscular injection. Other routes of  
administration were technically difficult because  
calcitonins were poorly absorbed through tissue and were  
readily degraded by bodily fluids. Despite these  
10 obstacles, a formulation (U.S. Patent 5,759,565) was  
developed that could be administered via the nasal route.  
The nasal formulation was designed to be stored in a  
multi-dose container that was stable for an extended  
period of time and resisted bacterial contamination. The  
15 preservative in the formulation, benzalkonium chloride,  
was found to enhance the absorption of salmon calcitonin.  
However, benzalkonium chloride was reported (P. Graf et  
al., Clin. Exp. Allergy 25:395-400; 1995) to aggravate  
rhinitis medicamentosa in healthy volunteers who were  
20 given a decongestant nasal spray containing the  
preservative. It also had an adverse effect on nasal  
mucosa (H. Hallen et al., Clin. Exp. Allergy 25:401-405;  
1995). Berg et al. (Laryngoscope 104:1153-1158; 1994)  
disclose that respiratory mucosal tissue that was exposed  
25 *in vitro* underwent severe morphological alterations.  
Benzalkonium chloride also caused significant slowing of

the mucociliary transport velocity in the ex vivo frog  
palate test (P.C. Braga et al., J. Pharm. Pharmacol.  
44:938-940; 1992).

#### SUMMARY OF THE INVENTION

5           Accordingly, the present invention provides a liquid  
pharmaceutical composition comprising calcitonin or an  
acid addition salt thereof and citric acid and/or salt  
thereof in a concentration from about 10 to about 50 mM,  
said composition being in a form suitable for nasal  
10       administration.

          The present invention also provides a liquid  
pharmaceutical composition comprising about 2,200 MRC  
units of salmon calcitonin, about 10 mM citric acid,  
about 0.2% phenylethyl alcohol, about 0.5% benzyl  
15       alcohol, and about 0.1% Tween 80.

          The present invention further provides a liquid  
pharmaceutical composition comprising about 2,200 MRC  
units of salmon calcitonin, about 20 mM citric acid,  
about 0.2% phenylethyl alcohol, about 0.5% benzyl  
20       alcohol, and about 0.1% Tween 80.

          The present invention also provides a method of  
administering a calcitonin to a subject requiring

calcitonin treatment, which method comprises  
administering via the nasal route to said subject a  
liquid pharmaceutical composition comprising calcitonin  
or an acid addition salt thereof and citric acid or salt  
5 thereof in a concentration from about 10 to about 50 mM.

The present invention further provides a method of  
improving the stability of a liquid pharmaceutical  
composition of calcitonin comprising adding citric acid  
or a salt thereof in a concentration from about 10 to  
10 about 50 mM to said composition.

The present invention also provides a method of  
improving the bioavailability or the concentration of  
plasma calcitonin in a subject following nasal  
administration of a liquid pharmaceutical composition of  
15 calcitonin, which method comprises adding citric acid or  
a salt thereof in a concentration from about 10 to about  
50 mM to said composition prior to said administration.

#### **DETAILED DESCRIPTION OF THE INVENTION**

In accordance with the present invention it has now  
20 been surprisingly found that pharmaceutical compositions  
can be obtained comprising a calcitonin as active  
ingredient which meet the high standards of stability and  
bioavailability required for nasal application and which

are, for example, eminently suitable for use in multiple dose nasal spray applicators, i.e., applicators capable of delivering a series of individual dosages over, e.g., period of several days or weeks, by the use of citric acid or a salt thereof in concentrations ranging from about 10 to about 50 mM as a buffering agent.

Surprisingly, it has also been found that use of citric acid or a salt thereof at increasing concentrations confers beneficial advantages in relation to the nasal absorption characteristics of calcitonin containing compositions and hence enhance calcitonin bioavailability levels consequential to nasal application. In addition, it has also been found that the use of citric acid or a salt thereof in concentrations ranging from about 10 to about 50 mM increase the stability of calcitonin containing compositions while at the same time higher concentrations of citric acid or salt thereof did not have the same stabilizing effect.

The calcitonins for use in the invention may be in free form or in pharmaceutically acceptable salt or complex form, e.g. in pharmaceutically acceptable acid addition salt form. Such salts and complexes are known and possess an equivalent degree of activity and

tolerability to the free forms. Suitable acid addition salt forms for use in accordance with the invention include for example the hydrochlorides and acetates.

5 The above defined compositions may be applied in accordance with the invention to the nasal mucosa, e.g. either in drop or in spray form. As hereinafter described however, they are most preferably applied in spray form, i.e., in the form of finely divided droplets.

10 The compositions of the invention may of course also include additional ingredients, in particular components belonging to the class of conventional pharmaceutically applicable surfactants. In this connection it has in accordance with a further aspect of the present invention been found that the use of surface active agents  
15 generally in relation to the nasal application of calcitonins, in particular salmon calcitonin, may increase absorption via the nasal mucosa and hence improve obtained bioavailability rates.

20 Preferably, the liquid pharmaceutical calcitonin composition of the present invention contains a pharmaceutically acceptable, a liquid diluent or carrier suitable for application to the nasal mucosa, most preferably aqueous saline.

The compositions of the invention are formulated so as to permit administration via the nasal route. For this purpose they may also contain, e.g. minimum amounts of any additional ingredients or excipients desired, for example, additional preservatives or, e.g. ciliary stimulants such as caffeine.

Generally for nasal administration a mildly acid pH will be preferred. Preferably the compositions of the invention have a pH of from about 3 to 5, more preferably from about 3.5 to about 3.9 and most preferably 3.7. Adjustment of the pH is achieved by addition of an appropriate acid, such as hydrochloric acid.

The compositions of the invention should also possess an appropriate isotonicity and viscosity. Preferably they have an osmotic pressure of from about 260 to about 380 mOsm/liter. Desired viscosity for the nasal spray is preferably less than 0.98 cP.

Compositions in accordance with the present invention may also comprise a conventional surfactant, preferably a non-ionic surfactant.

When a surfactant is employed, the amount present in the compositions of the invention will vary depending on

the particular surfactant chosen, the particular mode of administration (e.g. drop or spray) and the effect desired. In general, however, the amount present will be of the order of from about 0.1 mg/ml to about 10 mg/ml, preferably about 0.5 mg/ml to 5 mg/ml and most preferably about 1 mg/ml.

The amount of calcitonin to be administered in accordance with the method of the invention and hence the amount of active ingredient in the composition of the invention will, of course, depend on the particular calcitonin chosen, the condition to be treated, the desired frequency of administration and the effect desired.

As indicated in the following examples, bioavailability for calcitonins, in particular salmon calcitonin, as determined in terms of blood-plasma concentration following nasal administration in accordance with the teachings of the present invention has been found to be surprisingly high.

For nasal administration in accordance with the present invention, treatment will therefore suitably comprise administration of dosages of from about 50 to about 400 MRC units, more preferably from about 100 to



about 200 MRC units at a frequency of from about once daily to about three times weekly. Conveniently dosages as aforesaid will be administered in a single application, i.e., treatment will comprise administration  
5 of single nasal dosages comprising about 50 to about 400 MRC units, preferably about 100 to about 200 MRC units, calcitonin. Alternatively such dosages may be split over a series of 2 to 4 applications taken at intervals during the day, the dosage at each application then comprising  
10 about 10 to about 200, preferably about 25 to about 100 MRC units.

The total composition quantity administered at each nasal application suitably comprises from about 0.05 to 0.15 ml, typically about 0.1 ml. Compositions for use in  
15 accordance with the invention accordingly suitably comprise from about 150 to about 8,000, preferably from about 500 to about 4,000, more preferably from about 500 to about 3,000, yet again more preferably from about 1,000 to about 2,500, and most preferably about 2,200 MRC  
20 units of calcitonin per ml.

For the purposes of nasal administration, the compositions of the invention will preferably be put up in a container provided with means enabling application of the contained composition to the nasal mucosa, e.g.

put up in a nasal applicator device. Suitable applicators are known in the art and include those adapted for administration of liquid compositions to the nasal mucosa in drop or spray form. Since dosaging with calcitonins should be as accurately controlled as possible use of spray applicators for which the administered quantity is susceptible to precise regulation will generally be preferred. Suitable administrators include, e.g. atomizing devices, e.g. pump-atomizers and aerosol dispensers. In the latter case, the applicator will contain a composition in accordance with the invention together with a propellant medium suitable for use in a nasal applicator. The atomizing device will be provided with an appropriate spray adaptor allowing delivery of the contained composition to the nasal mucosa. Such devices are well known in the art.

The container, e.g. nasal applicator, may contain sufficient composition for a single nasal dosaging or for the supply of several sequential dosages, e.g. over a period of days or weeks. Quantities of individual dosages supplied will preferably be as hereinbefore defined.

The stability of the compositions of the invention may be determined in conventional manner. As indicated hereinbelow, the calcitonin content of the compositions of the invention will degrade less than 50% in 15 days at

50°C as indicated by standard analytical tests.

EXPERIMENTAL DESIGN - METHODS OF ADMINISTERING NASAL  
CALCITONIN AND MEASUREMENT OF PLASMA CONCENTRATION

Female Wistar rats, weighing between 225 and 250 g  
5 are anesthetized with a combination of ketamine and  
xyalazine, and a cannula is inserted into the carotid  
artery. The cannula is fitted to a three-way valve  
through which blood is sampled and replaced with  
physiological saline containing heparin. Formulated  
10 salmon calcitonin (sCT) ( $5\mu\text{g}$  per  $25\ \mu\text{l}$ ) is administered  
intranasally through a micropipette tip that was inserted  
8 mm into the rat's nostril. For single-dose studies,  $5\ \mu\text{g}$   
of sCT was administered. In multiple dose studies,  
sCT was administered four times in a volume of  $25\ \mu\text{l}$  each  
15 at 0, 30, 60 and 90 minutes for a total dose of  $20\ \mu\text{g}$ .

In single-dose studies, blood samples are collected  
prior to dosing and at 5, 15, 30, 60 and 120 minutes  
after dosing. In multiple-dose studies, blood samples  
are collected prior to dosing and at 30, 60, 90, 120 and  
20 150 minutes after the administration of the first dose.  
Blood samples are always collected immediately before the  
administration of any additional costs.

Each sample (0.5 ml) of blood is collected into a

heparinized 1 ml syringes and then transferred to chilled  
1.5 ml polypropylene tubes containing 10  $\mu$ l of heparin  
(500 U per ml). The tubes are centrifuged at  
approximately 3000 rpm for 20 minutes at 2-8°C and the  
5 plasma supernatant is transferred to microcentrifuge  
tubes that were stored at -20°C. The concentration of  
sCT in plasma is determined by a competitive  
radioimmunoassay. Aliquots of the plasma samples and  
standards are incubated for 4 hours at room temperature  
10 with rabbit anti-sCT antibody. Subsequently,  $^{125}$ I-sCT is  
added and incubated overnight at 2-8°C. Antibody-bound  
 $^{125}$ I-sCT is isolated the next day by precipitating it with  
normal rabbit antiserum and goat anti-rabbit antibody.  
Radioactivity associated with the resulting pellets is  
15 measured with a gamma counter. The concentration of sCT  
in plasma is inversely proportional to the amount of  
radioactivity that was precipitated.

The values of Cmax are determined by inspection and  
the values for bioavailability (relative to an  
20 intravenous injection) are calculated from the areas  
under the curve that were obtained from plots of plasma  
sCT concentration as a function of time.

#### Example 1

The following study examines the effect of the

concentration of citric acid on the bioavailability and plasma concentration of nasally administered salmon calcitonin. Rats were administered intranasally as described previously 20 $\mu$ l of rsCT (200 $\mu$ g/ml) in 0.85% sodium chloride, 0.1% Tween 80, 0.2% phenylethyl alcohol, 0.5% benzyl alcohol and varying amounts of citric acid adjusted to pH 3.7 at t=0, 20, 60 and 90 minutes. Samples of blood were taken prior to the administration of rsCT at these time points as well as at t=120 and 150 minutes. The resulting plasma samples were analyzed for rsCT by radioimmunoassay. Maximum rsCT levels were detected at t=120 minutes. The results of this study as shown in Table 1 indicate that the bioavailability and peak concentration of rsCT was a function of the concentration of citric acid in the formulation.

TABLE 1

EFFECT OF THE CONCENTRATION OF CITRIC ACID ON THE  
BIOAVAILABILITY AND PLASMA CONCENTRATION OF  
SALMON CALCITONIN ADMINISTERED INTRANASALLY TO RATS

5	Citric acid (pH 3.7) mM	Bioavailability (percent $\pm$ sdev)	Maximum plasma sCT (ng/ml $\pm$ sdev)
	0	0.89 $\pm$ 0.19	1.10 $\pm$ 0.52
	10	3.14 $\pm$ 1.77	3.66 $\pm$ 1.67
	25	5.01 $\pm$ 2.34	5.11 $\pm$ 2.09
10	50	6.15 $\pm$ 1.31	6.05 $\pm$ 1.30
	100	13.36 $\pm$ 3.38	12.98 $\pm$ 3.96

Example 2

The following study examines the effect of different  
preservatives on the plasma concentration of nasally  
15 administered salmon calcitonin. Rats were administered  
intranasally as described previously 20 $\mu$ l of sCT  
(200 $\mu$ g/ml) in 0.85% sodium chloride, 0.1% Tween 80 and a  
combination preservatives of either 0.2% phenylethyl  
alcohol and 0.5% benzyl alcohol or 0.27% methyl parabens  
20 and 0.04% propyl parabens at t=0, 30, 60 and 90 minutes.  
The results of this study as shown in Table 2 indicate  
that the bioavailability and peak concentration of rsCT  
are not significantly affected by the addition of the  
different preservatives.

TABLE 2  
EFFECT OF PRESERVATIVES ON THE  
AVAILABILITY AND PLASMA CONCENTRATION  
OF SCT ADMINISTERED INTRANASALLY TO RATS

5	Preservatives	Bioavailability (percent $\pm$ sdev)	Maximum plasma SCT (ng/ml $\pm$ sdev)
	None	1.14 $\pm$ 0.87	1.24 $\pm$ 0.79
	0.2% phenylethyl alcohol - 0.5% benzyl alcohol	0.89 $\pm$ 0.19	1.10 $\pm$ 0.52
10	0.27 methyl parabens - 0.04% propyl parabens	1.08 $\pm$ 0.86	1.47 $\pm$ 1.46

Example 3

The following study examines the effect of the concentration of citric acid on the stability of salmon calcitonin stored for varying periods at a temperature of 50°C. Nasal formulations containing SCT (200 $\mu$ g/ml), 0.25% phenylethyl alcohol, 0.5% benzyl alcohol and 0.1% Tween 80 were adjusted to pH 3.7 with either HCl or the indicated amount of buffered citric acid. The formulations were stored at 50°C in sealed glass containers for the indicated amount of time and analyzed for SCT by high performance liquid chromatography. The results as shown in Table 3 indicate that in the absence of citric acid, the amount SCT in the formulation decreased steadily between 0 and 9 days

after the study was begun. In the presence of citric acid (10-50 mM) the rate of disappearance of sCT decreased significantly. However, as the concentration of citric acid was further increased, the rate of sCT disappearance from vials stored at 50°C increased in proportion to the amount of buffered citric acid in the formulation.

TABLE 3  
EFFECT OF THE CONCENTRATION OF  
CITRIC ACID ON THE STABILITY OF  
sCT STORED FOR VARYING PERIODS AT 50°C

Percent sCT Recovered					
Citric Acid (pH 3.7)	0 mM	10 mM	20 mM	50 mM	100 mM
Days at 50°C					
0	100	100	100	100	100
3	83	94	91	90	87
6	53	90	87	83	77
9	24	82	78	73	66
15	22	74	68	61	20